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# *Borrelia* Species in Host-Seeking Ticks and Small Mammals in Northern Florida

Kerry Clark*

*Department of Public Health, University of North Florida, Jacksonville, Florida*

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**The aim of this study was to improve understanding of several factors related to the ecology and environmental risk of *Borrelia* infection in northern Florida. Small mammals and host-seeking adult ticks were collected at several sites, and specimens were tested for the presence of *Borrelia* species, primarily by PCR amplification. Tissues from some vertebrates and ticks were initially cultured in BSK-H medium to isolate spirochetes, but none were recovered. However, comparison of partial flagellin (*flaB*), 66-kDa protein (*p66*), and outer surface protein A (*ospA*) gene sequences from DNAs amplified from small mammals and ticks confirmed the presence of several *Borrelia* species. *Borrelia lonestari* DNA was detected among lone star ticks (*Amblyomma americanum*) at four sites. *Borrelia burgdorferi* sensu stricto strains were detected in all small mammal species tested and in *A. americanum*, *Ixodes affinis*, and *Ixodes scapularis* ticks. *Borrelia bissettii* was found in a cotton mouse and cotton rats and in *I. affinis* ticks. The study findings extend the known geographic distributions of *B. lonestari* in *A. americanum* and of *B. burgdorferi* sensu lato in *A. americanum*, *I. affinis*, *I. scapularis*, and small mammals to new sites in Florida. The presence of *B. burgdorferi* sensu stricto strains in host-seeking lone star ticks at two sites in Florida suggests that *A. americanum* should still be considered a possible vector of *B. burgdorferi* sensu lato.**

Lyme disease (LD), the most prevalent arthropod-borne disease in the United States, is caused by several species of spirochete bacteria within the *Borrelia burgdorferi* sensu lato genogroup (19). These species are maintained in nature and transmitted to humans by ticks of the genus *Ixodes*. Clinical symptoms affect all age groups and may involve the skin, joints, nervous system, and heart (49). *B. burgdorferi* sensu lato includes at least 10 genospecies, 3 of which (*B. burgdorferi* sensu stricto, *Borrelia andersonii*, and *Borrelia bissettii*) are present in North America (27, 41). In the northeastern United States, *B. burgdorferi* sensu stricto is the most common species, but it also occurs in western and southern states (28, 36, 41). Only *B. burgdorferi* sensu stricto has been proven to cause human disease in the United States. However, some *B. bissettii*-like strains may also be pathogenic (39, 51). There is significant genetic diversity among *B. burgdorferi* sensu lato strains and species in North America, particularly in areas other than the Northeast (4, 26, 28).

Most of what is known about the ecology of *B. burgdorferi* sensu lato in the eastern United States was derived from studies conducted in the Northeast, where the majority of human cases have occurred. There, *B. burgdorferi* sensu stricto is transmitted to humans by the blacklegged tick, *Ixodes scapularis* (6), and maintained in nature primarily by the white-footed mouse (*Peromyscus leucopus*) (25). Nevertheless, since the disease has become reportable, hundreds of cases have been reported from southeastern states, including Florida, Georgia, and South Carolina (9). Some of these cases may have resulted from exposures that occurred elsewhere in the country; however, many were locally transmitted (33). *B. burgdorferi* sensu lato (including *B. burgdorferi* sensu stricto) has been isolated from birds, rodents, and ticks in Florida, Georgia, South Carolina, and other southern states (12, 34, 36), but despite the information gathered to date, it is still unclear whether endemic human infection with *B. burgdorferi* sensu lato commonly occurs in the southern United States. Although LD incidence rates show that human risk is significantly lower in the Southeast than the Northeast, the underlying reasons for this are not well understood.

Complicating our understanding of LD in the Southeast is the emergence of a southern tick-associated rash illness (STARI) resembling the presentation of LD (8, 18, 23). STARI, also known as Master's disease, is associated with bites from the lone star tick, *Amblyomma americanum* (18, 23). Lone star ticks from several states in the eastern United States contained spirochetes that were noncultivable in BSK medium, which is typically used to isolate *B. burgdorferi* sensu lato, and are more closely related to relapsing fever *Borrelia* species (5, 7, 50, 52). This spirochete was named *Borrelia lonestari* (5).

The purpose of this study was to improve understanding of several factors related to the ecology and environmental risk of *Borrelia* infection in a large region of northern Florida. Specific objectives were to clarify the presence and distribution of *Borrelia* species among host-seeking ticks and small mammals; to identify, investigate genetic variability of, and characterize *Borrelia* strains by using molecular techniques; and to estimate the prevalence of infection with distinct *Borrelia* species among ticks and small mammals at selected study sites.

## MATERIALS AND METHODS

**Study area and localities.** Ticks were collected primarily at public recreation areas (state parks, wildlife management areas, and national forests) located in the northeastern region of Florida. The major terrestrial habitat types at those sites are pine flatwoods, mixed hardwood forest, coastal maritime hammock, high pine, and scrub (31). Small mammal sampling was conducted at two sites.

* Mailing address: Department of Public Health, University of North Florida, 4567 St. Johns Bluff Rd., Jacksonville, FL 32224. Phone: (904) 620-2840. Fax: (904) 620-2848. E-mail: kclark@unf.edu.

The University of North Florida Wildlife Sanctuary (UNFWS), located on the main campus in southeast Jacksonville, is a fragmented island of several hundred acres of natural habitat surrounded by development. The primary habitats are mesic to hydric mixed pine flatwoods (dominated by longleaf and slash pine, loblolly bay, and saw palmetto) and xeric to mesic mixed pine and oak uplands (dominated by longleaf and slash pine, turkey and live oak, scrub oak, and saw palmetto). The Guana River State Park and Wildlife Management Area (GRSPWMA) is located 30 miles south of Jacksonville and is a larger area (several thousand acres) bordered by the Atlantic Ocean on the east and by residential development on the west. It contains diverse and abundant wildlife and three distinct habitats: xeric to mesic maritime hammock (live oak, hickory, pines, holly, magnolia, and saw palmetto), mesic to hydric mixed pine flatwoods, and xeric to mesic mixed oak scrub (live oak, other oaks, and saw palmetto).

**Vertebrate and tick sampling.** Small mammals were captured live in Sherman traps baited with wild birdseed set in line transects in different habitat types at UNFWS and GRSPWMA between April and September 1999 and from July through September 2000. Captured animals were anesthetized by ketamine hydrochloride-xylazine injection, weighed, measured, and sexed. Ectoparasites were removed and preserved in ethanol for identification as part of a related study. A sample (~100 μl) of whole blood was collected via tail clip on Nobuto filter paper strips (Advantec MFS, Inc., Pleasanton, Calif.), allowed to dry, and stored under refrigeration until used for DNA extraction. The ears of captured animals were moistened with 70% ethanol and allowed to dry prior to removal of three 2-mm punches of tissue from each ear by using a rodent ear tag punch. Punches from one ear were placed in a 70:30 solution of sterile phosphate-buffered saline–glycerol and stored frozen for DNA extraction. Other samples were stored under refrigeration for no more than a few days prior to use in *Borrelia* isolation attempts. After examination and full recovery, animals were returned to their capture site. All procedures involving trapping and sampling of vertebrates were conducted in accordance with guidelines approved by the University of North Florida Institutional Animal Care and Use Committee and with permits from the Florida Department of Environmental Protection and Fish and Wildlife Conservation Commission.

Host-seeking ticks were collected by dragging 1-$m^2$ white felt flags along vertebrate trap transects, nature trails, and firebreaks at numerous study sites and removing ticks from clothing and the drag every ~15 m (every 15 to 20 paces). Most ticks were stored in ethanol for DNA extraction. Ticks destined for culture isolation were maintained live in vials with a few blades of fresh grass.

***Borrelia* isolation.** Attempts were made to isolate *Borrelia* from some vertebrate ear tissue samples and adult blacklegged ticks. Three ear punches from each rodent were removed from their transport vial; rinsed briefly in 10% povidone iodine, then in 70% ethanol, and then twice in sterile water; air dried; and finally placed in 4 ml of fresh BSK-H complete medium (Sigma, St. Louis, Mo.) supplemented with antibiotics (3). Live ticks were likewise surface sterilized, placed in a microtube with 200 μl of fresh medium, and ground with a sterile disposable pestle. Half of the suspension was inoculated into a fresh tube with 4 ml of medium. The other half was frozen for DNA extraction to compare the sensitivity of culture with that of DNA amplification. Samples from *B. burgdorferi* sensu lato reference strains (*B. burgdorferi* sensu stricto B31, JD1, NC92, and WI90; *Borrelia* sp. strain SCW-30H; and *B. andersonii* MOK-1C) were also inoculated to ensure the ability of the medium to support spirochete growth. Cultures were incubated at 33°C and examined for spirochetes by dark-field microscopy weekly for 4 weeks.

**DNA extraction.** All DNA extractions were conducted within a class II biological safety cabinet (NuAire, Plymouth, Minn.) used only for this purpose. DNA was extracted from host-seeking ticks, vertebrate ear tissue punches, Nobuto blood samples, and culture samples by using the DNeasy tissue kit (Qiagen, Valencia, Calif.) with optimized modifications of the manufacturer's protocols for each starting material. Early in the study, some ticks of the same species from the same site were pooled for DNA extraction. Later, DNA was extracted from individual ticks. The amounts of template typically used for other sample types were two to three 2-mm ear punches from an individual animal, a 5- by 5-mm piece of blood-soaked Nobuto strip from an individual animal, or a 1-ml sample of culture. All samples were incubated in 100 μg of proteinase K-tissue lysis buffer; ticks and ear punch samples were incubated at 55°C overnight, and Nobuto fragment and culture samples were incubated for a minimum of 1 h. After binding to the spin column and washing twice, DNA was eluted from the columns in a final volume of 200 μl of buffer AE for all samples, including those for pooled or individual ticks.

**PCR testing.** Due to low *Borrelia* target gene copy numbers in the extracts from vertebrates and ticks and to extraneous products in single-reaction PCRs with a high number of cycles, DNA extracts from ticks and from animal tissue and blood samples were tested for most *Borrelia* target genes via nested PCR assays. Several primer sets for different genes were used (Table 1).

Lone star tick extracts were first tested with a nested PCR assay designed to amplify a portion of the highly conserved 41-kDa chromosomal flagellin (*flaB*) gene of *Borrelia* species (5). Flagellin-positive samples were then confirmed by testing with a *Borrelia* species-specific 16S rRNA gene (rDNA) primer set (42). Samples were initially screened for the presence of *B. burgdorferi* sensu lato DNA by using a different nested PCR designed to amplify a portion of the *flaB* gene of all *B. burgdorferi* sensu lato species (20). Most of the samples that tested positive in this assay were then tested with a nested PCR assay that amplifies a portion of the chromosomal 66-kDa protein (*p66*) gene of *B. burgdorferi* sensu lato in the United States (44). For this assay, a different inner reverse primer (*p66* inner 2 [Table 1]) was designed and used to amplify a 296-bp product. Many of the *B. burgdorferi* sensu lato *flaB*-positive samples were also tested with two nested PCR assays that target portions of the ~31-kDa outer surface protein A (*ospA*) gene of *B. burgdorferi* sensu lato (16, 17) and with a nested assay with primers targeting the intergenic spacer region (ISR) between the *rrf* (5S)-*rrl* (23S) rDNA (43).

Reaction mixtures for single-stage PCRs and first-round amplifications of nested PCR assays contained between 2.5 and 5 μl of DNA extract per individual sample in a total reaction volume of 50 μl. Extracts from individual ticks from some sites were initially screened in pools of three for efficiency. All reactions utilized a hot start master mix (TaKaRa *Taq* HS; PanVera Corp., Madison, Wis.), resulting in final concentrations of 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate, 1.25 U of *Taq* polymerase, and 0.5 μM each primer, and were carried out in an automated DNA thermal cycler (Geneamp PCR System 2400 [Perkin-Elmer, Norwalk, Conn.] or PTC 200 [MJ Research, Watertown, Mass.]). Single-stage and outer PCRs consisted of initial denaturation at 95°C for 1 min, followed by 40 cycles of 94°C for 30 s, primer annealing at the temperature listed in Table 1 for 30 s, and extension at 72°C for 1 min. Mixtures for nested reactions included between 1 and 2.5 μl of outer reaction product as the template for another 30 cycles with the same parameters and annealing temperature profile as described above and in Table 1.

PCRs were set up in a separate area within PCR clean cabinets (CleanSpot workstation [Coy Laboratory Products, Grass Lake, Mich.] or PCR workstation [CBS Scientific, Del Mar, Calif.]) equipped with germicidal UV lamps. Other precautions to prevent carryover contamination of amplified DNA included different sets of pipettes dedicated for DNA extraction, PCR setup, and post-amplification activities; the use of aerosol barrier filter pipette tips; and exposure of PCR tubes, pipettes, and tips to UV light prior to PCR setup. Each PCR included a negative control sample with sterile water as template and a positive control sample with *B. andersonii* (MOK-1C) culture extract.

Amplicons were visualized on 2% agarose gels stained with ethidium bromide and were documented with a digital gel imaging system (GelDocMega; BioSystematica, Devon, United Kingdom).

**DNA purification and sequencing.** PCR-amplified gene fragments were purified of primers and other nonspecific amplification by-products by using the QIAquick PCR purification kit (Qiagen) and were sequenced for species confirmation and phylogenetic comparison. Because of the large number of amplicons analyzed in this study, samples were sequenced in only one direction, using the nested forward primer for each target gene fragment. DNA templates were sequenced by using the fluorescent dideoxy terminator method of cycle sequencing on either a Perkin-Elmer Applied Biosystems (ABI) 373A or 377 automated DNA sequencer, according to ABI protocols (29). Sequences were generated by using Sequencher software (Gene Codes Corporation, Ann Arbor, Mich.).

**Sequence analysis.** Investigator-derived sequences were compared with those obtained by searching the GenBank database (National Center for Biotechnology Information) with the Basic Local Alignment Search Tool (1) and were aligned by using Clustal X (54). The GenBank accession numbers used for comparison with the *B. lonestari flaB* gene sequences reported in this study are AF264901, AF273670, AF298653, AF408410, D43777, D82859, D82861, D82862, D82863, D82864, D86618, U26704, U26705, U28498, U28499, X15661, X75202, and X75204. The accession numbers used for comparison with the *B. burgdorferi* sensu lato *flaB* gene sequences reported here are AB035595, AF264883, AF264886, AF264889, AF264892, AF264894, D82847, D82849, D82852, D82854, D82856, D82857, D83762, D83763, L29245, U26704, X16933, X75200, X75202, and X75203. The accession numbers used for comparison with the *B. burgdorferi* sensu lato *p66* gene sequences are AE001161, AY090473, U96240, U96241, U96243, and X87727, and those used for comparison with the *B. burgdorferi* sensu lato *ospA* gene sequences are A24008, AB016975, AF186846, AF369944, AY030279, L23144, X80257, U20360, U65802, X16467, Y10838, Y10840, Y10892, Y10897, and Z29087. Phylogenetic trees were constructed by

TABLE 1. Oligonucleotide primers used in this study

| Species and target gene | Primer | Primer sequence (5′→3′) | Base position | Annealing temp (°C) | Amplicon size (bp) | Reference |
|---|---|---|---|---|---|---|
| *Borrelia* spp. | | | | | | |
| 16S rDNA | BF1 | GCT-GGC-AGT-GCG-TCT-TAA-GC | 41–60 | 58 | 1,351 | 42 |
| | BR1 | GCT-TCG-GGT-ATC-CTC-AAC-TC | 1391–1371 | | | 42 |
| Flagellin gene | FlaLL | ACA-TAT-TCA-GAT-GCA-GAC-AGA-GGT | 301–324 | 55 | 665 | 5 |
| | FlaRL | GCA-ATC-ATA-GCC-ATT-GCA-GAT-TGT | 965–942 | | | 5 |
| | FlaLS | AAC-AGC-TGA-AGA-GCT-TGG-AAT-G | 438–459 | 55 | 354 | 5 |
| | FlaRS | CTT-TGA-TCA-CTT-ATC-ATT-CTA-ATA-GC | 791–766 | | | 5 |
| *B. burgdorferi* | | | | | | |
| Flagellin gene | Outer 1 | AAG-TAG-AAA-AAG-TCT-TAG-TAA-GAA-TGA-AGG-A | 245–275 | 55 | 611 | 20 |
| | Outer 2 | AAT-TGC-ATA-CTC-AGT-ACT-ATT-CTT-TAT-AGA-T | 855–825 | | | 20 |
| | Inner 1 | CAC-ATA-TTC-AGA-TGC-AGA-CAG-AGG-TTC-TA | 300–328 | 55 | 390 | 20 |
| | Inner 2 | GAA-GGT-GCT-GTA-GCA-GGT-GCT-GGC-TGT | 689–663 | | | 20 |
| *p66* | Outer 1 | CGA-AGA-TAC-TAA-ATC-TGT | 147–164 | 37 | 371 | 44 |
| | Outer 2 | GAT-CAA-ATA-TTT-CAG-CTT | 517–500 | | | 44 |
| | Inner 1 | TGC-AGA-AAC-ACC-TTT-TGA-AT | 166–188 | 42 | 296 | 44 |
| | Inner 2 | GCT-GCT-TTT-GAG-ATG-TGT-CC | 461–442 | | | This study |
| *ospA* | Outer 1 | AAA-AAA-TAT-TTA-TTG-GGA-ATA-GG | 4–26 | 42 | 702 | 16 |
| | Outer 2 | GTT-TTT-TTG-CTG-TTT-ACA-CTA-ATT-GTT-AA | 695–667 | | | 16 |
| | Inner 1 | GGA-GTA-CTT-GAA-GGC-G | 220–235 | 42 | 345 | 16 |
| | Inner 2 | GCT-TAA-AGT-AAC-AGT-TCC | 564–547 | | | 16 |
| *ospA* | N1 | GAG-CTT-AAA-GGA-ACT-TCT-GAT-AA | 334–356 | 42 | 561 | 17 |
| | C1 | GTA-TTG-TTG-TAC-TGT-AAT-TGT | 894–874 | | | 17 |
| | N2 | ATG-GAT-CTG-GAG-TAC-TTG-AA | 362–381 | 42 | 352 | 17 |
| | C2 | CTT-AAA-GTA-ACA-GTT-CCT-TCT | 713–693 | | | 17 |
| 5S-23S spacer | Outer 1 | TAA-GCT-GAC-TAA-TAC-TAA-TTA-CCC | 92–115 | 50 | 377 | 43 |
| | Outer 2 | ACC-ATA-GAC-TCT-TAT-TAC-TTT-GAC | 469–446 | | | 43 |
| | Inner 1 | GAG-AGT-AGG-TTA-TTG-CCA-GGG | 243–263 | 55 | 225 | 43 |
| | Inner 2 | ACC-ATA-GAC-TCT-TAT-TAC-TTT-GAC-CA | 469–444 | | | 43 |

using the neighbor-joining (NJ) and unweighted pair-group method with arithmetic mean (UPGMA) distance methods and by parsimony analysis (46, 53). Tree topologies and evolutionary relationships obtained with the different methods were compared for consensus. The tree-building program was MEGA 2.1 (24). To estimate the node reliability of trees obtained with each method, bootstrap values (13) based on an analysis of 1,000 replicates were determined. Distance matrices were generated by the methods of Jukes and Cantor (21) and by the Kimura two-parameter model for multiple substitutions (22).

**Nucleotide sequence accession numbers.** The GenBank accession numbers for the *B. lonestari flaB* gene sequences reported in this study are AY654941 to AY654945, those for the *B. burgdorferi* sensu lato *flaB* gene sequences reported here are AY654901 to AY654918 and AY654946, those for the *B. burgdorferi* sensu lato *p66* gene sequences are AY654926 to AY654940, and those for the *B. burgdorferi* sensu lato *ospA* gene sequences are AY654919 to AY654925.

## RESULTS

***Borrelia* isolation.** Early in the study, attempts were made to isolate *Borrelia* from vertebrate ear tissue samples from a small number of animals ($n = 22$) and from adult blacklegged ticks ($n = 72$) from UNFWS and GRSPWMA. The animals included 1 golden mouse, 18 cotton mice, and 3 rice rats. No viable spirochetes were observed in the vertebrate or tick specimen cultures, despite PCR amplification of various *B. burgdorferi* sensu lato target gene fragments from the ear tissue extracts from most of the vertebrates (see below) and from a few of the tick extracts and excellent growth of *B. burgdorferi* sensu lato reference strain cultures inoculated into aliquots of the same medium and maintained under identical conditions. Because of the lack of agreement between culture isolation results and PCR, no further attempts were made to isolate *Borrelia* in this study.

***Borrelia* species-specific PCR.** *Borrelia* sp. *flaB* DNA that was determined to be from *B. lonestari* (see below) was detected in eight samples from host-seeking adult lone star ticks from four study sites, three in northeastern Florida and one in the north-central part of the state (Table 2 and Table 3; Fig. 1). A few of the positive samples were from ticks extracted and tested as a pool, and a few were from extracts from single ticks (Table 2). Each positive tick pool was treated as if it contained a single positive tick. All of the *Borrelia* sp. *flaB* PCR-positive lone star tick samples also tested positive with *Borrelia* sp.-specific 16S rDNA primers. The *B. lonestari* infection prevalence among ticks at individual sites ranged from 0 to 4.8%. The overall prevalence among 396 ticks was 2.0% (Table 3).

***B. burgdorferi* sensu lato-specific PCR.** *B. burgdorferi* sensu lato *flaB* DNA was amplified from DNA extracts from seven small mammal and three tick species in Florida (Table 2, Table 4, and Table 5). Approximately 85% (56 of 66) of small vertebrates tested positive (Table 4), including two new hosts recorded for *B. burgdorferi* sensu lato: the southern flying squirrel (*Glaucomys volans*) and the golden mouse (*Ochrotomys nuttalli*). The infection prevalences were very similar among

TABLE 2. *Borrelia* strains identified in the present study

| Strain name | Host (number and stage) | Locality in Florida | Genospecies[a] |
|---|---|---|---|
| AA4POOL | *A. americanum* (8 adults) | Alexander Springs, Lake County | *B. burgdorferi* sensu stricto |
| AA15POOL | *A. americanum* (8 adults) | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| AA15POOL | *A. americanum* (8 adults) | UNFWS, Duval County | *B. lonestari* |
| AA16POOL | *A. americanum* (8 adults) | UNFWS, Duval County | *B. lonestari* |
| AA17POOL | *A. americanum* (7 adults) | UNFWS, Duval County | *B. lonestari* |
| AA18POOL | *A. americanum* (3 adults) | GRSPWMA, St. Johns County | *B. lonestari* |
| AA97 | *A. americanum* (adult) | O'Leno State Park, Columbia County | *B. lonestari* |
| AA115 | *A. americanum* (adult) | GRSPWMA, St. Johns County | *B. lonestari* |
| AA116 | *A. americanum* (adult) | GRSPWMA, St. Johns County | *B. lonestari* |
| AA207 | *A. americanum* (adult) | Tomoka State Park, Volusia County | *B. lonestari* |
| AA31-33POOL | *A. americanum* (3 adults) | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| AA10-12POOL | *A. americanum* (3 adults) | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| AA139-141POOL | *A. americanum* (3 adults) | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| FLAS1 | *I. scapularis* (adult) | Alexander Springs, Lake County | *B. burgdorferi* sensu stricto |
| FLBT6 | *I. scapularis* (adult) | Big Talbot Island, Duval County | *B. burgdorferi* sensu stricto |
| FLCL3 | *I. affinis* (adult) | Clearwater Lake, Lake County | *B. burgdorferi* sensu stricto |
| FLFD1 | *I. scapularis* (adult) | Faver-Dykes State Park, St. Johns County | *B. burgdorferi* sensu stricto |
| FLFG14 | *I. affinis* (adult) | Fort George Island, Duval County | *B. burgdorferi* sensu stricto |
| FLGR7 | *I. scapularis* (adult) | GRSPWMA, St. Johns County | *B. burgdorferi* sensu stricto |
| FLGR11 | *I. scapularis* (adult) | GRSPWMA, St. Johns County | *B. burgdorferi* sensu stricto |
| FLGR-C3 | *I. scapularis* (adult) | GRSPWMA, St. Johns County | *B. burgdorferi* sensu stricto |
| FLJS14 | *I. affinis* (adult) | Juniper Springs, Marion County | *B. bissettii* |
| FLLS1 | *I. scapularis* (adult) | Lower Suwanee NWR, Levy County | *B. burgdorferi* sensu stricto |
| FLNF26 | *I. affinis* (adult) | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| FLNF27 | *I. affinis* (adult) | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| FLSF1 | *I. scapularis* (adult) | Stephen Foster Folk Center, Columbia County | *B. burgdorferi* sensu stricto |
| FLTP1 | *I. scapularis* (adult) | Tomoka State Park, Volusia County | *B. burgdorferi* sensu stricto |
| IA1 | *I. affinis* (adult) | Alexander Springs, Lake County | *B. bissettii* |
| KC1 | *Peromyscus gossypinus* | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| KC9 | *Ochrotomys nuttalli* | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| KC10 | *Peromyscus gossypinus* | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| KC14 | *Glaucomys volans* | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| KC18 | *Ochrotomys nuttalli* | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| KC19 | *Didelphis virginianus* | UNFWS, Duval County | *B. burgdorferi* sensu stricto |
| FL1 | *Ochrotomys nuttalli* | GRSPWMA, St. Johns County | *B. burgdorferi* sensu stricto |
| FL5 | *Peromyscus gossypinus* | GRSPWMA, St. Johns County | *B. burgdorferi* sensu stricto |
| FL8 | *Oryzomys palustris* | GRSPWMA, St. Johns County | *B. burgdorferi* sensu stricto |
| FL9 | *Oryzomys palustris* | GRSPWMA, St. Johns County | *B. burgdorferi* sensu stricto |
| FL18 | *Peromyscus gossypinus* | UNFWS, Duval County | *B. bissettii* |
| FL27 | *Sigmodon hispidus* | GRSPWMA, St. Johns County | *B. bissettii* |
| FL35 | *Sigmodon hispidus* | GRSPWMA, St. Johns County | *B. bissettii* |
| FL42 | *Sigmodon hispidus* | GRSPWMA, St. Johns County | *B. bissettii* |

[a] Genospecies of *B. burgdorferi* sensu lato were determined by *flaB*, *p66*, and *ospA* sequences; *B. lonestari* was determined by *flaB* sequences.

vertebrates at both sites. PCR-positive DNA extracts were obtained from vertebrate ear tissue and Nobuto blood samples. Thirty of 40 *flaB*-positive mammal ear tissue samples (75%) tested positive with the *p66* assay also. However, only a smaller fraction (12 of 37; 32%) of *flaB*-positive samples also tested positive with either set of *ospA* or the 5S-23S ISR primers. These results were consistent upon retesting of many of the samples several times with each primer set. Experimentation with different amounts of starting template and modifications of PCR amplification parameters did not vary the results.

*B. burgdorferi* sensu lato *flaB* DNA was detected in five extracts from host-seeking adult lone star ticks from two sites (Table 2 and Table 5). One of the pooled samples (AA15POOL) tested positive for both *B. lonestari* and *B. burgdorferi* sensu stricto (Table 2; Fig. 2 and 4). The overall prevalence among all lone star ticks tested from four sites was 2.0% (Table 5). Because *Ixodes affinis* adults were collected in only small numbers at any given site, all ticks tested from 10 different sites were combined to estimate the infection prevalence in that species, which was nearly 31% (Table 5). Positive *I. affinis* ticks came from five sites in Florida and one in southeastern Georgia (Cumberland Island, Camden County). *B. burgdorferi* sensu lato *flaB* DNA was detected in adult *I. scapularis* from all 13 sites in northern Florida from which ticks were tested (Table 2; Fig. 1). Enough ticks were tested from two coastal sites to estimate the infection prevalence, which was 4.6% among 216 ticks (Table 5).

***Borrelia* species flagellin sequences.** Florida *B. lonestari flaB* sequences, consisting of approximately 320 nucleotides of data, were more than 99% similar to all *B. lonestari* sequences in GenBank and clustered with other *B. lonestari* sequences in the phylogenetic trees produced by various tree-building methods (Fig. 2). All of the Florida sequences were identical to the sequence with accession number AF298653 from a lone star tick in Alabama (7) and to two sequences derived from a previously reported human patient and an attached lone star tick (accession numbers AF273670 and AF273671, respectively) (18). The sequence from the amplicon produced with

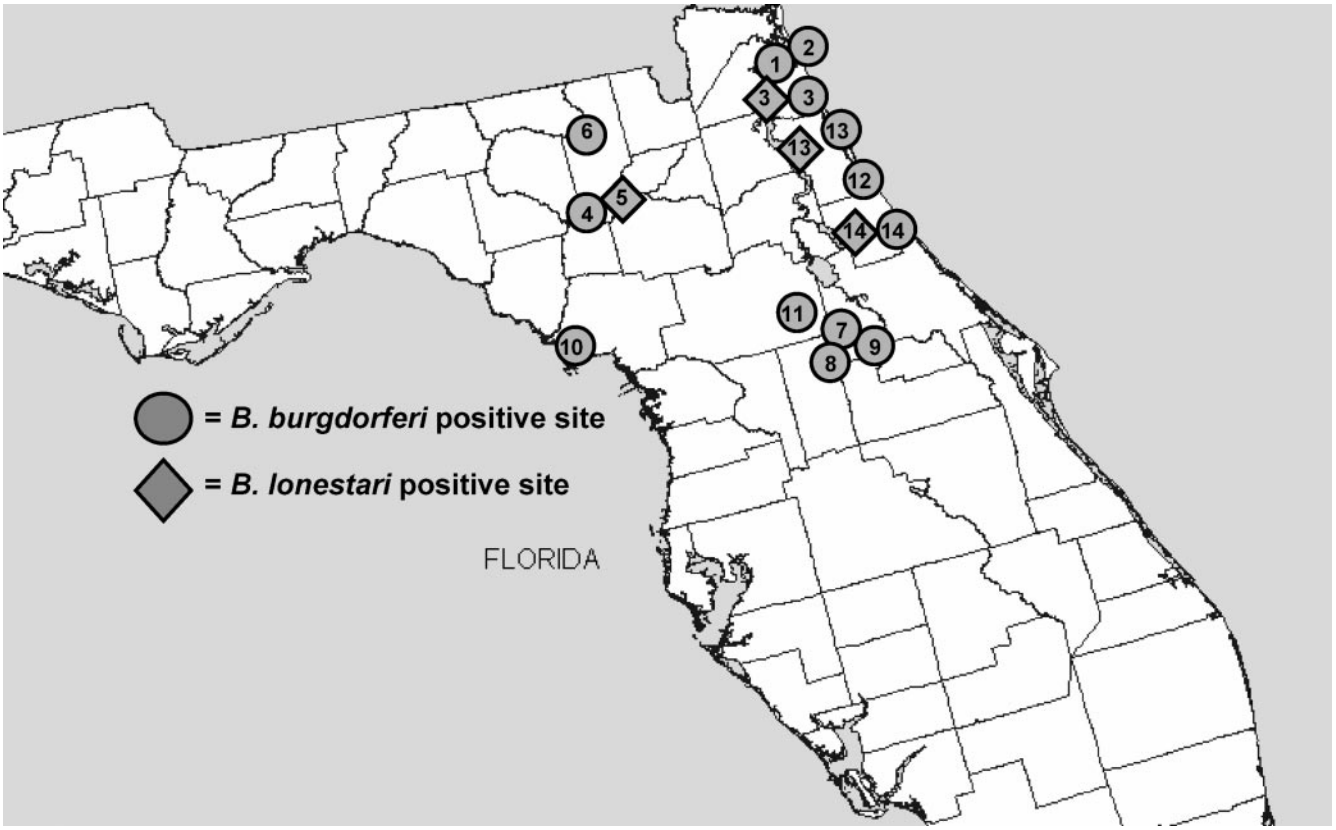


FIG. 1. Map of Florida showing geographic distribution of *Borrelia* species detected in ticks via nested flagellin gene PCR. Circles indicate locations of study sites with *B. burgdorferi* sensu lato-positive blacklegged ticks, *I. affinis*, or lone star ticks. Triangles indicate locations of study sites with *B. lonestari*-positive lone star ticks. 1, Big Talbot Island State Park, Duval County; 2, Fort George Island, Duval County; 3, UNFWS, Duval County; 4, Ichetucknee Springs State Park, Columbia County; 5, O'Leno State Park, Columbia County, 6, Stephen Foster State Folk Center, Columbia County; 7, Alexander Springs, Lake County; 8, Clearwater Lake, Lake County; 9, River Forest, Lake County; 10, Lower Suwanee National Wildlife Refuge, Levy County; 11, Juniper Springs, Marion County; 12, Faver-Dykes State Park, St. John's County; 13, GRSPWMA, St. Johns County; 14, Tomoka State Park, Volusia County.

the *Borrelia* sp.-specific *flaB* primers for the Florida AA4POOL sample was 99.1% similar to the sequence for *B. burgdorferi* sensu stricto B31 and clustered with it in the phylogenetic tree (Fig. 2). All other lone star tick samples that produced *flaB* sequences similar to those of the *B. burgdorferi* sensu lato complex were obtained with the *B. burgdorferi* sensu lato-specific PCR primers.

***B. burgdorferi* sensu lato flagellin, *p66*, and *ospA* sequences.** Florida *B. burgdorferi* sensu lato *flaB* amplicons derived from several different small mammal and tick species extracts were sequenced, and the sequences were compared to *B. burgdorferi* sensu lato reference strain sequences. Approximately 362 nucleotides of data were compared. The phylogenetic trees created by different methods were very similar. All of the Florida *flaB* sequences clustered with reference strains of either *B. burgdorferi* sensu stricto or *B. bissettii* (Fig. 3). Sequences that clustered with *B. burgdorferi* sensu stricto reference strains were obtained from all vertebrate and tick species tested (Table 2; Fig. 3), including lone star ticks. A smaller number of Florida sequences clustered with *B. bissettii* reference strains. Sequences that clustered in this group were obtained from cotton mice, cotton rats, a rice rat, and *I. affinis* (not all shown in Fig. 3).

TABLE 3. Prevalence of *B. lonestari* flagellin DNA among lone star ticks collected in Florida, 1999 to 2000

| County | Site | No. ticks tested/ no. positive | Infection prevalence (%) |
|---|---|---|---|
| Columbia | O'Leno State Park | 1/36 | 2.8 |
| Duval | UNFWS | 3/118 | 2.5 |
| Lake | Alexander Springs | 0/27 | 0 |
| | River Forest | 0/35 | 0 |
| Marion | Juniper Springs | 0/36 | 0 |
| St. Johns | GRSPWMA | 3/63 | 4.8 |
| Suwanee | Stephen Foster State Park | 0/36 | 0 |
| Volusia | Tomoka State Park | 1/45 | 2.2 |
| Total | | 8/396 | 2.0 |

The phylogenetic trees constructed with *p66* sequences also clustered Florida sequences with either *B. burgdorferi* sensu stricto or *B. bissettii* reference strains. Figure 4 shows the NJ tree obtained with *p66* sequences. In this analysis, *B. bissettii* 25015 was located most closely on the tree to *B. andersonii* MOK-1c, rather than being clustered with other *B. bissettii* strains from Florida and Colorado. Another difference in comparison to the *flaB* trees was that the sequence for strain SCW-30 h, isolated from an *I. minor* tick in South Carolina

TABLE 4. Prevalence of *B. burgdorferi* sensu lato flagellin DNA among small mammals collected in Florida, 1999 to 2000

| County | Site | No. of PCR positive animals/no. tested (% positive)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Virginia opossum | Flying squirrel[b] | Golden mouse[b] | Rice rat | Cotton mouse | Cotton rat | Wood rat | Total |
| Duval | UNFWS | 1/1 (100) | 1/1 (100) | 2/2 (100) | | 27/30 (90) | 2/2 (100) | | 33/36 (92) |
| St. Johns | GRSPWMA | 0/1 (0) | | 1/1 (100) | 3/3 (100) | 9/10 (90) | 9/13 (69) | 1/2 (50) | 23/30 (77) |
| Total | | 1/2 (50) | 1/1 (100) | 3/3 (100) | 3/3 (100) | 36/40 (90) | 11/15 (73) | 1/2 (50) | 56/66 (85) |

[a] Cotton mouse, *Peromyscus gossypinus;* cotton rat, *Sigmodon hispidus;* flying squirrel, *Glaucomys volans;* golden mouse, *Ochrotomys nuttalli;* rice rat, *Oryzomys palustris;* Virginia opossum, *Didelphis virginianus;* wood rat, *Neotoma floridana.*
[b] New host for *B. burgdorferi* sensu lato.

(26), was not placed near *B. burgdorferi* sensu stricto strains. The NJ and UPGMA bootstrap consensus trees (not shown) and the maximum-parsimony tree (data not shown) also placed the SCW-30h *p66* sequence on a separate branch from all other *B. burgdorferi* sensu lato strains included in the analysis. However, based on the *flaB* sequence analysis, this strain is most similar to *B. burgdorferi* sensu stricto and *B. bissettii*.

None of the Florida *ospA* sequences analyzed in this study represented strains of *B. burgdorferi* sensu stricto; all clustered with *B. bissettii* reference strains (Fig. 5). The phylogenies obtained with *ospA* sequences were very similar regardless of the tree construction method and generally are in agreement with those derived from analysis of *flaB* and *p66* sequences. For example, based on *ospA* sequence data, *B. bissettii* 25015 clustered with other *B. bissettii* strains, and SCW-30h clustered with *B. burgdorferi* sensu stricto strains, albeit somewhat distantly (Fig. 5). The primary difference between the *flaB* and *ospA* phylogenies for strains of *B. burgdorferi* sensu lato analyzed in this study was that the *B. andersonii ospA* sequences were most similar to a those of a strain of *Borrelia valaisiana* (VS116), while *B. andersonii flaB* sequences were most similar to those of *B. burgdorferi* sensu stricto.

## DISCUSSION

DNA amplification and sequence analysis showed that most of the *B. burgdorferi* sensu lato strains identified in this study from small mammals and ticks were very similar to *B. burgdorferi* sensu stricto, including strains identified in the animals and ticks whose tissues were cultured but from which no spirochetal isolates were recovered. *ospA*- and 5S-23S ISR-positive samples in this study clustered with reference strains of *B. bissettii* in the phylogenetic trees created with their flagellin, *p66*, and *ospA* sequences. Only the *B. bissettii* strains identified in this study amplified a product of the expected size and sequence with the *ospA* and 5S-23S ISR primers used. All of the flagellin and *p66* sequences from *ospA*-negative samples clustered with *B. burgdorferi* sensu stricto strains. Probably all or nearly all of the other *ospA*-positive strains were also *B. bissettii* strains, and all or nearly all of the *ospA*-negative strains were most like *B. burgdorferi* sensu stricto. Based on this assumption, 79% (44 of 56) of the flagellin-positive small mammals were infected with *B. burgdorferi* sensu stricto, and 21% (12 of 56) were infected with *B. bissettii*. *B. bissettii* strains were identified in four vertebrate species: cotton rat ($n = 8$), golden mouse ($n = 1$), rice rat ($n = 2$), and cotton mouse ($n = 1$). Based on the amplicons sequenced in this study, the only tick species infected with *B. bissettii* was *I. affinis*. It is possible, however, that some PCR-positive *I. scapularis* ticks contained *B. bissettii* but that those amplicons were not sequenced. *B. burgdorferi* sensu stricto strains were found in all vertebrate species tested, as well as in host-seeking adult *A. americanum*, *I. affinis*, and *I. scapularis*.

TABLE 5. Prevalence of *B. burgdorferi* sensu lato flagellin DNA among host-seeking adult ticks collected in northern Florida and southeastern Georgia, 1999 to 2000

| County | Site | Tick species | No. ticks tested/ no. positive | Infection prevalence (%) |
|---|---|---|---|---|
| Duval | UNFWS | *A. americanum* | 4/118 | 3.4 |
| Lake | Alexander Springs | *A. americanum* | 1/27 | 3.7 |
| | River Forest | *A. americanum* | 0/35 | 0 |
| St. Johns | GRSPWMA | *A. americanum* | 0/63 | 0 |
| Species total | | *A. americanum* | 5/252 | 2.0 |
| Several[a] | Several[a] | *I. affinis* | 20/65 | 30.8 |
| Duval | UNFWS | *I. scapularis* | 5/108 | 4.6 |
| St. Johns | GRSPWMA | *I. scapularis* | 5/108 | 4.6 |
| Species total | | *I. scapularis* | 10/216 | 4.6 |

[a] Includes ticks from 10 sites, 9 in Florida and 1 in Georgia (Cumberland Island, Camden County).

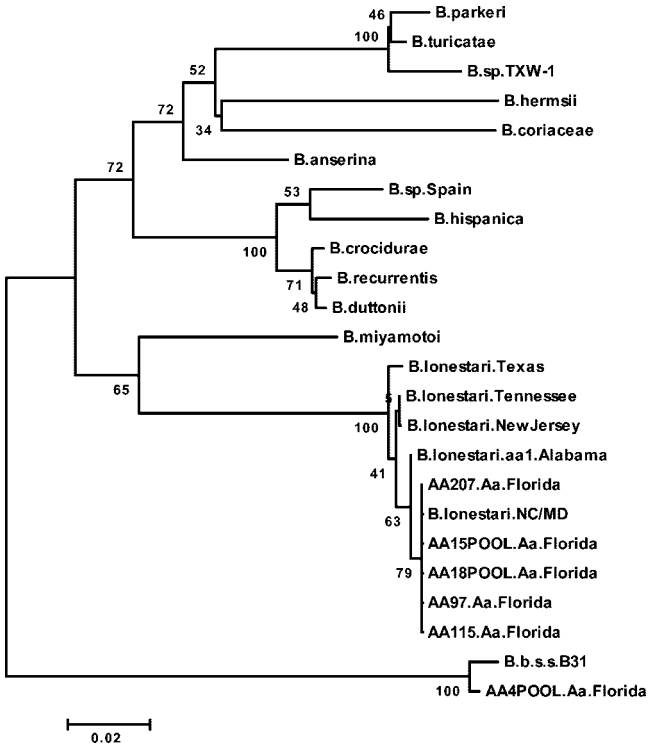


FIG. 2. Unrooted neighbor-joining phylogenetic tree based on a comparison of partial flagellin gene sequences obtained from Florida lone star ticks with other *Borrelia* species. *B. burgdorferi* sensu stricto strain B31 was included as an outgroup. Numbers at the branch nodes represent bootstrap values as percentages of 1,000 replications.

The various *ospA* serotypes of *B. burgdorferi* sensu lato have been associated with different clinical manifestations of LD (58). Some *B. burgdorferi* sensu lato strains isolated in Europe (57) and several strains isolated from *I. scapularis* ticks removed from humans and vegetation in the northeastern United States (2) lacked *ospA*. However, most *B. burgdorferi* sensu stricto strains isolated in the United States express *ospA*, or some form of *ospA* or *ospB*, including those from the southeastern United States (36). The *ospA* sequences of distinct *B. burgdorferi* sensu lato species vary significantly, while those of most *B. burgdorferi* sensu stricto strains analyzed thus far are very homogeneous (28, 57).

In comparison, *B. andersonii* strains have shown significant heterogeneity in their reactivity to specific *ospA* monoclonal antibodies (35). Strain MOD-6, isolated from lone star tick larvae removed from a rabbit in Missouri, failed to react with *ospA* monoclonal antibodies H3TS and H5332, as well as monoclonal antibody H6831 for *ospB*, in a previous study (35). That strain also failed to amplify an *ospA* fragment with one of three different *ospA* PCR primer sets. Using the primers described in Table 1, it was not possible to amplify detectable *ospA* from any *B. burgdorferi* sensu stricto strain characterized in the present study. These southern *B. burgdorferi* sensu stricto strains may simply lack *ospA*, as do previously described strains from the northeastern United States (2). Another possible explanation is that *ospA* is expressed by these strains but the *ospA* genes of these strains differ significantly enough in the region of the primers used in the present study to prevent correct primer annealing or to reduce the sensitivity of amplification of the target gene fragments below the level needed to identify them via electrophoresis and UV transillumination, despite the use of highly sensitive nested PCR assays. Recombination between *ospA* and *ospB* proteins, resulting in the deletion of *osp* gene sequences and the creation of chimeric gene fusions, has been described for some *B. burgdorferi* sensu lato strains (45, 48).

The *ospA*-negative samples in the present study also failed to amplify a product in PCRs with the nested 5S-23S ISR PCR assay, suggesting the possibility of atypical differences in rRNA arrangement in these same strains. Such findings have been reported in other studies. For example, a *B. bissettii* 25015-like strain isolated from a patient in Slovenia failed to amplify a product with a different set of 5S-23S ISR primers (39). Two strains of *B. andersonii* (21038 and 19857) possess a single copy of 5S (*rrfA*) and an interrupted or fragmented second copy of 23S (*rrlB*), rather than the typical two complete copies of each, and a *B. japonica* strain (IKA2) contains only single copies of both 23S (*rrlA*) and 5S (*rrfA*) (27). The lack of a second copy of 23S (*rrlB*) prevented amplification of the IKA2 product with forward and reverse primers located within the two copies of 23S (*rrlA* and *rrlB*, respectively), but a product was amplified with primers located within the first copies of 23S and 5S (*rrlA* and *rrfA*) (27).

All of the *B. bissettii* strains identified in small mammals and ticks from Florida, the *B. andersonii* MOK-1c reference strain, and several *B. burgdorferi* sensu stricto reference strains amplified products of the expected size with the 5S-23S ISR and both *ospA* PCR assays used in this study, demonstrating the ability of the primers to amplify strains of genetically distinct species groups from many samples. Nevertheless, the *ospA*- and 5S-23S ISR-negative PCR results in this study could stem from inadequate sensitivity of those assays due to low copy numbers of the target genes in some experimental samples. Contamination of PCR samples does not explain the *flaB*- and *p66*-positive but *ospA*- and 5S-23S ISR-negative PCR results with *B. burgdorferi* sensu stricto strain samples from Florida, since *B. andersonii* MOK-1c was used as a positive control in the testing. Contamination of DNA extracts with reference strain culture sample DNA also cannot explain the findings, since none of the Florida *B. burgdorferi* sensu stricto *flaB* or *p66* sequences were identical to those of the cultured strains.

The patterns of PCR positivity and negativity observed in this study suggest that the predominant strains of *B. burgdorferi* sensu lato in the study region may comprise a more genetically distinct group of *B. burgdorferi* sensu stricto than has previously been described. This group of *B. burgdorferi* sensu stricto strains may be resistant to culture in BSK-H medium and be variable in *ospA* and 5S-23S rRNA gene expression and/or arrangement. Alternatively, these strains may be cultivable, but perhaps they result in very low spirochetemia, below the level of detection via isolation in BSK-H medium and DNA amplification with the *ospA* and 5S-23S ISR primers used in this study. Additional testing of the *ospA*- and 5S-23S ISR-negative samples from Florida with other primers may show whether *ospA* and rRNA variation similar to that for some *B. andersonii* or *B. japonica* strains explains the PCR results in the present study. If so, it will be interesting to learn whether such strains also exist elsewhere. Previous studies that relied on culture

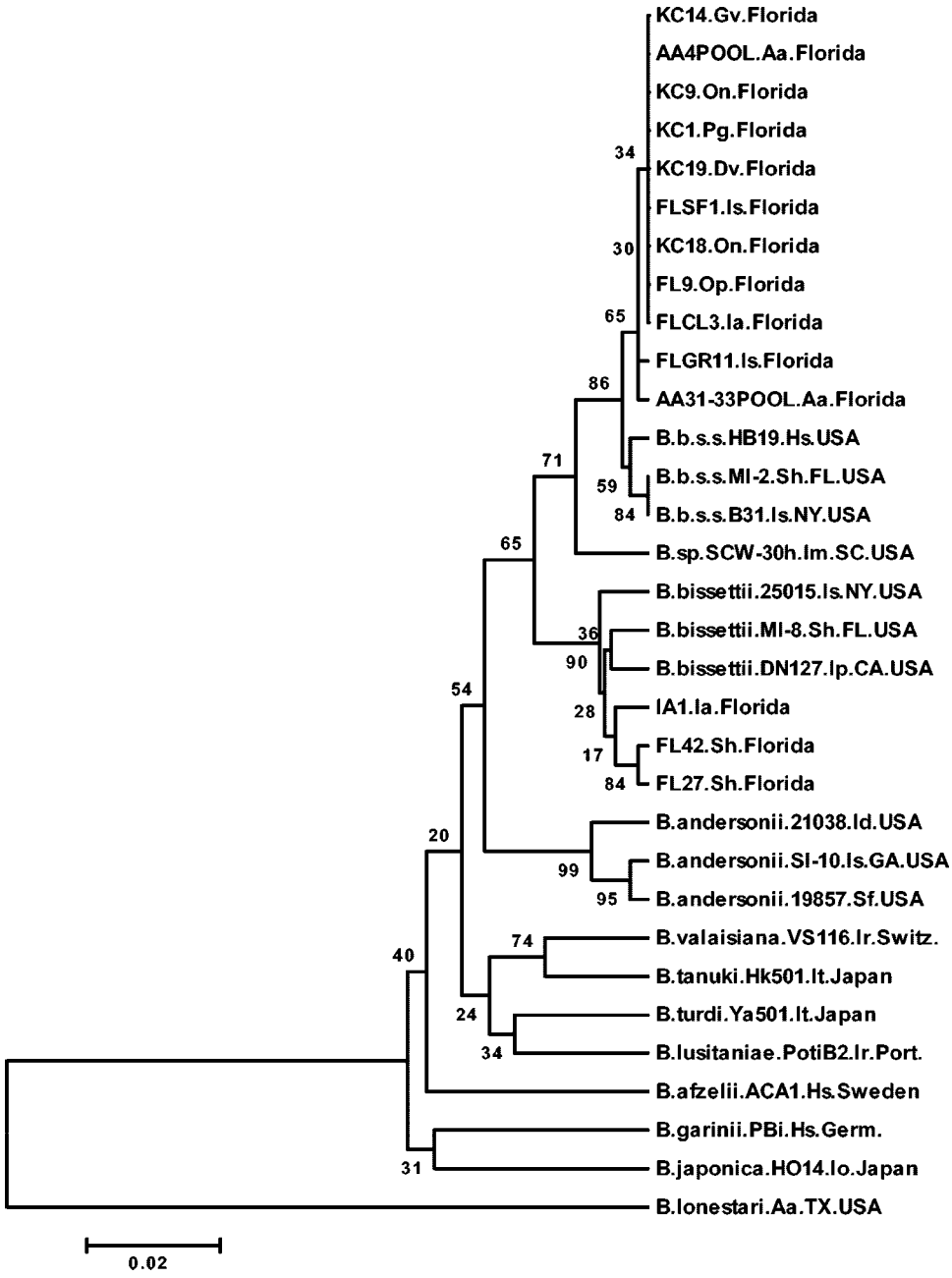


FIG. 3. Unrooted UPGMA phylogenetic tree based on a comparison of partial flagellin gene sequences obtained from Florida small mammals and ticks with other *B. burgdorferi* sensu lato species. *B. lonestari* was included as an outgroup. Numbers at the branch nodes represent bootstrap values as percentages of 1,000 replications.

isolation, *ospA* PCR assays, or 5S-23S ISR PCR testing for initial detection of *B. burgdorferi* sensu lato could have failed to recognize such strains present in the respective areas, just as the present study would have failed to identify them had it relied solely upon similar methods of detection. Even more intriguing are the potential human disease implications if such strains are capable of infecting humans and causing Lyme disease-like manifestations. Would human blood, tissues, or other specimens from patients infected with similar strains and tested with typical diagnostic tests, including antibody tests, culture, or PCR, produce positive results? Alternatively, these strains may not be infectious or pathogenic to humans.

Many spirochete species (e.g., *Treponema pallidum*) have proven difficult to cultivate. Previous efforts to isolate *Borrelia* spp. from lone star ticks in BSK medium failed. *B. lonestari* was identified and described based solely on DNA amplification and sequence analysis and was only recently isolated in a tick cell line (55). It has been demonstrated that isolation in BSK medium does not detect all genotypes of *B. burgdorferi* sensu lato circulating in a given area. The genetic diversity of *B.*

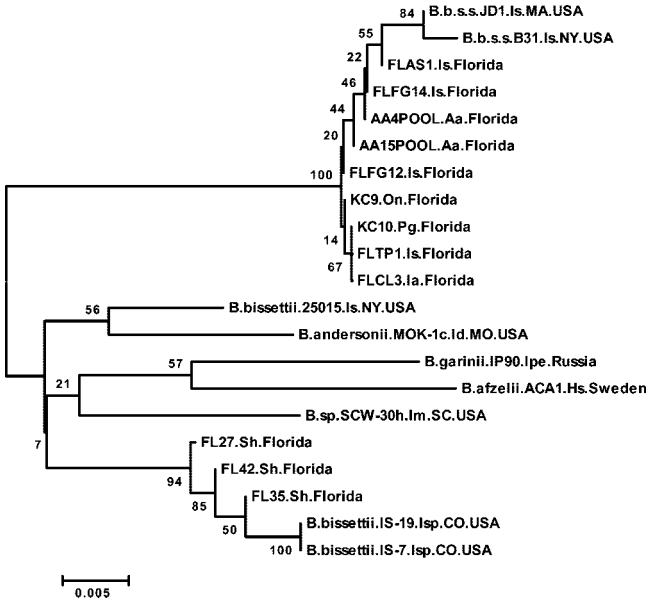


FIG. 4. Unrooted neighbor-joining phylogenetic tree based on a comparison of partial *p66* gene sequences obtained from Florida small mammals and ticks with other *B. burgdorferi* sensu lato species. Numbers at the branch nodes represent bootstrap values as percentages of 1,000 replications.

*burgdorferi* sensu lato detected in samples from humans, other vertebrates, and ticks via PCR amplification is greater than that detected by initial culture of spirochetes in BSK (30, 32). Even DNA testing via PCR amplification for detection, if based on amplifying some genes that vary considerably such as *ospA*, may not be adequately sensitive or reliable for detecting all *B. burgdorferi* sensu lato strains in an area.

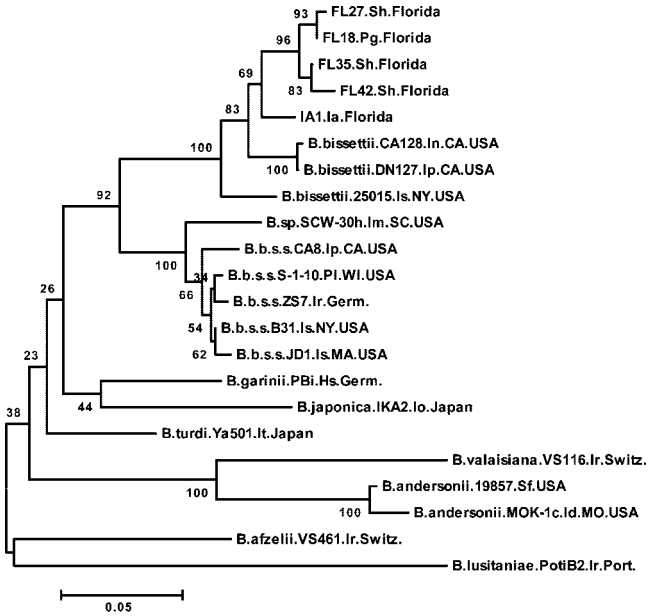


FIG. 5. Unrooted neighbor-joining phylogenetic tree based on a comparison of partial *ospA* gene sequences obtained from Florida small mammals and ticks with other *B. burgdorferi* sensu lato species. Numbers at the branch nodes represent bootstrap values as percentages of 1,000 replications.

The results of this study suggest that future studies aimed at identifying the full diversity of *B. burgdorferi* sensu lato strains in a given area should use highly sensitive, DNA amplification-based methods that target conserved genes. The nested *B. burgdorferi* sensu lato *flaB* PCR assay used in this study proved to be most reliable and identified strains present in ticks and small mammals that would not have been identified by the *p66* or other assays. Moreover, the *flaB*-based phylogeny in the present study and results of other flagellin-based typing systems (15, 38) have agreed very well with other *B. burgdorferi* sensu lato typing methods, including sequence analysis of different gene targets, PCR-based restriction fragment length polymorphism analysis, pulsed-field gel electrophoresis, and randomly amplified polymorphic DNA analysis (56).

The *B. lonestari* infection prevalence (2%) in lone star ticks in Florida is similar to that found in other states (5, 52). If *B. lonestari* strains in *A. americanum* in Florida are pathogenic to humans, then the risk for STARI (Master's disease) is present in the study area. Considering the feeding habits and regional abundance of lone star ticks, this could explain a significant portion of the cases of Lyme disease-like illness recognized in Florida. However, the discovery of *B. burgdorferi* sensu stricto strains in an equal proportion of lone star ticks, along with the genetic heterogeneity identified in the strains in Florida that belong to this group, contribute to ongoing suspicions of this tick's involvement in transmitting *B. burgdorferi* sensu lato to humans. Although they are much less aggressive in biting humans in this region, *I. scapularis* ticks are also infected with *B. burgdorferi* sensu lato strains and could occasionally transmit them to people. The prevalence of infection in adult *I. affinis* ticks in Florida based on PCR testing (31%) was similar to that found in South Carolina (25.7%) by using culture isolation (11). However, this tick species is not known to bite humans and probably is important only in the enzootic transmission of the spirochete among small mammals, which are the preferred hosts for the immature tick stages (10).

This study showed via PCR testing that 4.6% of adult *I. scapularis* ticks from two coastal sites in Florida contained *B. burgdorferi* sensu lato DNA. Only 1.3% in South Carolina were infected, based on BSK culture results (11). The present study also identified a very high *B. burgdorferi* sensu lato infection prevalence (85%) among small mammals from two sites near the Atlantic Coast in northeast Florida. This is higher than the rates determined for small mammals in South Carolina, Georgia, and Florida in previous studies (11, 37). Interestingly, if infection prevalence data from a previous study (37) for cotton mice, cotton rats, and wood rats, three established small mammal reservoir species in the Southeast, are combined from all sites within those three states and compared, a potential trend is apparent. The combined prevalence measures among animals of the three species tested from South Carolina, Georgia, and Florida are 41.8% (82 of 196), 12.0% (33 of 274), and 6.5% (13 of 200), respectively (37). Those data were based upon initial isolation of spirochetes in BSK. The numbers of each species tested from each state were not equal, and the samples were collected from different sites within each state. Therefore, sampling bias could explain some of the variation. The higher vertebrate infection prevalence found in the present study

compared to that for animals from Florida tested in the other study is most likely explained by my use of DNA amplification methods instead of culture isolation for detection of *B. burgdorferi* sensu lato. Taken together, these findings may be indicative of an actual north-south trend in the proportion of *B. burgdorferi* sensu lato strains that are cultivable in BSK rather than a trend in actual vertebrate infection prevalence. This supports a theory that the predominant *B. burgdorferi* sensu stricto strains in Florida (and perhaps in other southern states) may be difficult or impossible to cultivate in BSK. Comparisons of culture isolation and *flaB* PCR testing of samples from areas throughout the southeastern United States are needed to test this hypothesis.

Based on amplification and analysis of flagellin and *p66* gene fragments, the predominant strains of *B. burgdorferi* sensu lato identified in this study in Florida are *B. burgdorferi* sensu stricto. However, they may be quite different from reference strains of *B. burgdorferi* sensu stricto in their *ospA* and 5S-23S rRNA genes. It is not known whether the Florida strains are pathogenic to humans or, even if they are, whether they are transmitted to humans. If these strains are pathogenic but can be transmitted to humans only by *I. scapularis*, then probably only low numbers of humans in this area become exposed or infected each year. The nymphal and adult stages of this tick species do not frequently parasitize humans in this region (14).

The lone star tick, however, is extremely aggressive and at all life stages bites humans. *B. lonestari* is a suspected human pathogen, and it was found in ticks at several sites in Florida. Yet, several pools of host-seeking adult lone star ticks were also infected with *B. burgdorferi* sensu stricto strains that are identical in their *flaB* and *p66* sequences to the *B. burgdorferi* sensu stricto strains found in *I. scapularis* and small mammals in Florida. This may appear to disagree with the published findings that lone star ticks cannot acquire or maintain *B. burgdorferi* sensu lato This longstanding belief is based upon laboratory transmission studies conducted necessarily with reference strains of *B. burgdorferi* sensu stricto that were cultured or cultivable in BSK (40, 47). The *B. burgdorferi* sensu stricto strains described in this study are probably not identical to those, as described above, and evidence from this study suggests that they may not be as easily cultured, if cultivable at all, in BSK.

The findings of the present study extend the known geographic distributions of *B. lonestari* in *A. americanum* and of *B. burgdorferi* sensu lato in *A. americanum*, *I. affinis*, *I. scapularis*, and small mammals to new sites in northern and central Florida. They document new hosts for *B. burgdorferi* sensu lato infection, the flying squirrel and golden mouse, both of which may serve as additional reservoirs for the bacteria in the study area. The presence of *B. burgdorferi* sensu stricto strains in host-seeking lone star ticks at two sites in Florida suggests that *A. americanum* should still be considered a possible vector of at least some *B. burgdorferi* sensu lato strains. The implications of this study's findings for the human risk of infection in the southeastern United States with previously uncharacterized *B. burgdorferi* sensu lato strains or species or those not presently considered to be human pathogens, as well as the possibility of Lyme disease spirochete transmission from lone star ticks in addition to *I. scapularis*, demonstrate the need for further investigation of the ecology and epidemiology of borreliosis in the southern United States.

## ACKNOWLEDGMENTS

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